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(57) Abstract

This invention relates to the field of pharmaceuticals. More specifically, this invention relates to novel water-soluble esters of probucol for administration as antioxidants and pro-drugs of probucol. More specifically, the present invention relates to pharmaceutical formulations comprising novel water-soluble phosphate esters of probucol.

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PROBUCOL ESTERS AND USES THEREOF

The present application is a Continuation-In-Part application ("CIP") of U.S. Provisional Application No. 60/065,267, filed November 10, 1997. The aforementioned application is explicitly incorporated herein by reference in its entirety and for all purposes.

FIELD OF THE INVENTION

This invention relates to the field of pharmaceuticals. More specifically, this invention relates to novel water-soluble esters of probucol for administration as antioxidants and pro-drugs of probucol. More specifically, the present invention relates to pharmaceutical formulations comprising novel water-soluble phosphate esters of probucol.

BACKGROUND OF THE INVENTION

Probucol is a water-insoluble antioxidant. Probucol is structurally related to the widely used food additives 2,[3]-tert-butyl-4-hydroxyanisole (BHA) and 2,6-di-tert-butyl-4- methylphenol (BHT). Probucol has also been used as a pharmaceutical agent to treat a variety of infections, traumas, and pathologic conditions, including, *e.g.*, to lower serum cholesterol levels in hypercholesterolemic patients (Zimetbaum (1990) *J. Clin. Pharmacol.* 30:3-9; Schwartz (1988) *Am. J. Cardiol.* 62:1B05B; Buckley (1989) *Drugs* 37:761-800); diabetic cardiomyopathy (Kaul (1996) *Mol. Cell. Biochem.* 160-161:283-8); femoral atherosclerosis (Regnstrom (1996) *Atherosclerosis* 125:217-29), xanthelasma (Fujita (1996) *J. Dermatol.* 23:598-602), restenosis (Lee (1996) *Jpn. Heart J.* 37:327-32), tumorigenesis (Zarkovic (1995) *Carcinogenesis* 16:2599-601), puromycin nephrosis (Magil (1996) *J. Am. Soc. Nephrol.* 7:2340-7), and HIV infection (Hendler, et al., U.S. Patent No. 4,985,465), smoke inhalation damage (Ishizaki (1996) *Clin. Sci. (Colch)* 90:517-23). The strong antioxidant activity of probucol is thought to be linked to many of its pharmacological activities (Bisby (1996) *Free Radic. Biol. Med.* 20:411-20; Kuzuya (1993) *Free Radic. Biol. Med.* 14:67-77).

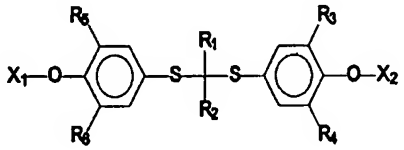
Probucol is commonly administered in the form of tablets typically containing celluloses and other excipients. Administration of the tablet form of probucol can present difficulties because probucol is absorbed at significantly different rates and in different amounts by different patients (see, *e.g.*, Heeg (1980) *La Nouvelle Presse Medicale*

9(40):2990-4). Some success in finding an alternative to tablet delivery of probucol has been achieved by use of a solution of probucol in a triglyceride oil. This formulation can enhance systemic absorption of the drug (Sanchez, *et al.*, U.S. Patent 5,494,936). However, because probucol is both highly crystalline and highly lipophilic, which makes it almost totally insoluble in water, it is poorly absorbed into the blood, and much of it is excreted in a substantially unchanged form. Thus, this extremely low solubility of probucol hinders its therapeutic efficacy and limits the therapeutic applications of the drug. Unsuccessful attempts have been made to solve these problems by converting probucol to water-soluble pro-drug derivatives; see, *e.g.*, Parthasarathy, *et al.*, U.S. Patent 5,262,439. Because probucol has these various problems, an alternative means of delivery, or formulations of probucol, having improved absorption characteristics or stability properties, would be highly desirable.

In view of the foregoing, the need exists for a stable water-soluble pharmaceutical preparation having the therapeutic properties of probucol, which is readily and predictably absorbed by the body to maximize the benefit of its administration. The present invention fulfills these and other needs.

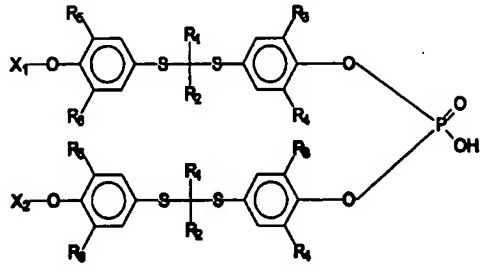
SUMMARY OF THE INVENTION

The invention provides a probucol ester of the formula:



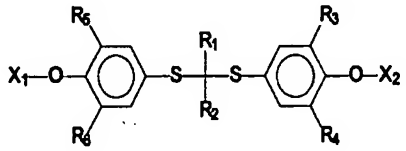
wherein X_1 is H- or - PO_3-H_2, and X_2 is H- or - PO_3-H_2 ; wherein R_1 and R_2 are H- or -CH_3; and R_3, R_4, R_5, and R_6 are independently selected from H- or an alkyl group selected from the group consisting of methyl, ethyl, propyl, butyl or tert-butyl; or, a pharmaceutically acceptable salt thereof. In a preferred embodiment, R_3, R_4, R_5, and R_6 are tert-butyl groups.

The invention also provides a diprobucol ester of the formula



wherein X_1 is H- or - PO_3-H_2, and X_2 is H- or - PO_3-H_2 ; wherein R_1 and R_2 are H- or -CH_3; and R_3, R_4, R_5, and R_6 are independently selected from H- or an alkyl group selected from the group consisting of methyl, ethyl, propyl, butyl or tert-butyl; or, a pharmaceutically acceptable salt thereof. In a preferred embodiment, R_3, R_4, R_5, and R_6 are tert-butyl groups.

The invention provides for a lithium probucol compound of the formula:



wherein X_1 is a lithium, and X_2 is H- or a lithium; wherein R_1 and R_2 are H- or -CH_3; and R_3, R_4, R_5, and R_6 are independently selected from H- or an alkyl group selected from the group consisting of methyl, ethyl, propyl, butyl or tert-butyl; or, a pharmaceutically acceptable salt thereof. In a preferred embodiment, R_3, R_4, R_5, and R_6 are tert-butyl groups.

The invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a pharmacologically effective amount of a probucol ester or diprobucol ester of the invention. In alternative embodiments, the pharmaceutical composition can be in a unit dosage form; the unit dosage form can contain between about 0.1 mg per kg to about 1 gm per kg, between about 1.0 mg per kg to about 100 mg per kg, or between about 10 mg per kg to about 50 mg per kg . The pharmaceutical composition can be in an aqueous solution, a solid, a liposome, a transdermal carrier, a detergent or an emulsifier. It can be formulated as a powder, a tablet, a capsule or a lozenge. The pharmaceutical composition can be administered by intramuscular, intravenous, intraperitoneal (I.P.), intrathecal, intradermal, oral, rectal, nasal, ocular, transdermal, or topical routes, or as an inhalant.

In one embodiment, the pharmaceutical composition is in the form of an injectable solution, and the concentration of the compound in the injectable solution can be between about 1 mg/ml to about 100 mg/ml. In alternative embodiments, the pharmaceutical composition can be contained in an aerosolizer or inhaler, or can be contained in a transdermal delivery system.

The invention also provides for a method for inhibiting oxidation or oxidative damage in a biological material or an organism comprising administering to the material or organism an effective amount of a probucol ester of the invention. The biological material can be a biological solution, a cell, or a tissue. The organism can be a plant, an animal, or a human. The effective amount can be between about 0.1 mg per kg to about 1 gm per kg, between about 1.0 mg per kg to about 100 mg per kg, between about 10 mg per kg to about 50 mg per kg; or between about 1.0 mg per kg to about 1 gm per kg . In the method of the invention the pharmaceutical composition can be administered intramuscularly, intravenously, intraperitoneally, intrathecally, intradermally, orally, rectally, nasally, ocularly, transdermally, topically or as an inhalant. The methods include prophylactic or therapeutic treatment of a condition with an inflammatory component in a subject, comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. The probucol ester can be administered in the form of an intravenous injection of a pharmaceutical formulation and a pharmaceutically acceptable aqueous carrier. In alternative embodiments, the concentration of the probucol ester in the aqueous composition is between about 1 mg/ml to about 100 mg/ml; and is between about 1 mg/ml to about 20 mg/ml. Oxidation or oxidative damage can be mediated by one or more enzymes, *e.g.*, myeloperoxidase, superoxide dismutase, nitric oxide synthase and the oxidative repiratory burst enzymes neutrophils and macrophages. Oxidation or oxidative damage in a biological material or an organism can also be mediated by drugs, infectious agents, toxins, pollutants, heavy metals or radiation.

The invention provides a method for the prophylactic or therapeutic treatment of a viral infection in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. The viral infection can be, but is not limited to, a herpes virus infection, a cytomegalovirus infection, a retroviral infection, or a human immunodeficiency virus infection. The retroviral infection can be an HIV-1 infection. The viral infection can be sexually or non-sexually transmitted. In one embodiment of this method, the compound is applied topically or is administered *ex vivo*, as in the *ex vivo* treatment of cells, such as, *e.g.*, lymphocytes. In one embodiment, the method

is for the treatment of a viral infection in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention, wherein said administering to the subject comprises the *ex vivo* treatment of cells, such as, *e.g.*, lymphocytes.

The invention provides a product of manufacture for the prophylactic treatment of a viral infection in a subject comprising a pharmacologically effective amount of a probucol ester of the invention. In different embodiments, the product of manufacture can be a lubricant for sexual intercourse, a condom, or other prophylactic device.

The invention provides a method for the prophylactic or therapeutic treatment of reperfusion injury in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. The reperfusion injury can be caused by - a consequence of - a cerebrovascular accident ("stroke"), myocardial infarction or trauma.

The invention provides a method for the prophylactic or therapeutic treatment of restenosis in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. The restenosis can occur after angioplasty (*e.g.*, percutaneous transluminal coronary angioplasty or PTCA), coronary bypass surgery, cardiovascular surgery or a lytic procedure (*e.g.*, streptokinase treatment).

The invention provides a method for the prophylactic or therapeutic treatment of hypercholesterolemia in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention.

The invention provides a method for the inhibition of the oxidation of low density lipoproteins in a subject, comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention

The invention provides a method for the prophylactic or therapeutic treatment of a peripheral neuropathy in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention.

The invention provides a method for the prophylactic or therapeutic treatment of a pathologic condition of the central nervous system (CNS) in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. In various embodiments, the pathological CNS condition can be caused by the neurotoxicity effected by oxidative stress or beta-amyloid neurotoxicity. The condition can be, *e.g.*, Alzheimer's Disease, traumatic brain injury, traumatic spinal chord injury, Parkinson's disease, Huntington's disease, Pick's disease, and dementias in general.

The invention provides a method for the prophylactic or therapeutic treatment of an opthalmic disorder in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. Without limitation, the opthalmic disorder can be macular degeneration.

The invention provides a method for the prophylactic or therapeutic treatment of a hearing disorder in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. Without limitation, the hearing disorder can be noise-induced hearing loss.

The invention provides a method for the prophylactic or therapeutic treatment of a pulmonary disorder in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. Without limitation, the pulmonary disorder can be asthma, acute respiratory distress syndrome (ARDS), or chronic obstructive pulmonary disease (COPD).

The invention provides a method for the prophylactic or therapeutic treatment of a cardiovascular disease in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. Without limitation, the cardiovascular disease can be reperfusion injury, atherosclerosis, cerebrovascular accident ("stroke"), restenosis, myocardial infarction ("heart attack"), or elevated cholesterol or LDL oxidation.

The invention provides a method for inhibiting cholesterol oxide formation in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention.

The invention provides a method for the inhibition of macrophage foam cell formation in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention.

The invention provides a method for the inhibition of oxysterol synthesis in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. The oxysterol can be 7 beta-hydroxycholesterol or 7-ketocholesterol.

The invention provides a method for the inhibition of oxidative damage (*i.e.*, peroxidation) associated with diabetes mellitus in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention

The invention provides a method for the prophylactic or therapeutic treatment of a drug-induced oxidative stress in a subject comprising administering to the subject a pharmacologically effective amount of a probucol ester of the invention. In different embodiments, the drug can be a chemotherapeutic agent or an antitumor drug, and, without limitation, the chemotherapeutic agent can be bleomycin or adriamycin.

The invention provides a method for the treatment of a cancer in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the cancer both alone and in combination with chemotherapeutic agents, and also in combination with radiotherapeutic agents.

The invention provides a method for the treatment of an arthritis in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the arthritis. Without limitation, the arthritis can be rheumatoid arthritis, psoriatic arthritis, or osteoarthritis.

The invention provides for a method for the treatment of pre-eclampsia and eclampsia in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the pre-eclampsia or eclampsia.

The invention provides for a method for the treatment of infertility due to, amongst other things, increased oxidative stress in a male or female subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the infertility.

The invention provides for a method for the treatment of endometriosis.

The invention provides for a method for the treatment of erectile dysfunction.

The invention provides a composition comprising a bio-organic material and an amount of a probucol ester of the invention effective to inhibit oxidation of the bio-organic material. The bio-organic material can be, *e.g.*, a blood plasma preparation, a nutrient media, a protein, a pharmaceutical, a cosmetic, a sperm or a oocyte preparation, cells or a cell culture, a virus, a food, a drink, an implant material or an implantable device, a medical materials or a container for bio-organic material.

A further understanding of the nature and advantages of the present invention is realized by reference to the remaining portions of the specification, the figures and claims.

All publications, patents and patent applications cited herein are hereby expressly incorporated by reference for all purposes.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

To facilitate understanding the invention, a number of terms are defined below. Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology* (2d ed. 1994); *The Cambridge Dictionary of Science and Technology* (Walker ed., 1988); and Hale & Marham, *The Harper Collins Dictionary of Biology* (1991). Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The term "biological material" refers to bioorganic molecules, cells, tissues, organs, organisms, extracts, homogenates, fluids or cultures, both *in vitro* and *in vivo*.

The term "condition" refers to any physiologic state that is not optimally normal or healthy, including, *e.g.*, an injury, infection, disease, pathology, drug side effect, contamination (as *e.g.*, a pollutant), poisoning, irritation, predisposition (*e.g.*, as in a genetic predisposition) or abnormal blood chemistry (*e.g.*, elevated cholesterol or elevated LDL).

The term "inflammatory component" as used herein incorporates its common usage and refers to that component of an injury, disease or condition involving the immune response, particularly, the damaging oxidating compositions generated directly or indirectly by an immune reaction/response. As explained in further below, many, if not most, diseases, conditions and injuries involve an inflammatory component which contributes directly or indirectly to pathogenesis and tissue damage. The probucol esters of the invention, as anti-oxidants, can ameliorate and prevent these deleterious inflammatory insult(s).

The term "oxidative stress" refers to an imbalance between production of reactive oxygen species and antioxidant protection resulting in overproduction of at least one reactive oxygen species, including, *e.g.*, superoxide anion, hydroxyl radical, hydrogen peroxide, peroxynitrite, hypochlorite, or alkylperoxide. An individual can be clinically evaluated to determine the relative state of oxidative stress by measuring and monitoring serum levels of, *e.g.*, malondialdehyde (MDA) (an indicator of cell membrane lipid peroxidation); isoprostanes; oxidized glutathione; glutathione peroxidase; superoxide

dismutase (SOD) or catalase. An example of the use of MDA analysis to measure lipid oxidation resulting from oxidative stress induced by, *e.g.*, angina, is given by Kostner (1997) *Cardiovascular Res.* 36:330-336; see also, Gambhir (1997) *Clin. Biochem.* 30:351-355; Proudfoot (1997) *Free Radic. Biol. Med.* 23:699-705. Objective measures of oxidative stress include detection of serum levels of TBARS (thiobarbituric acid reaction substances), isoprostanes, 8-hydroxy-2-deoxyguanosine, protein bound acrolein, methionine sulfoxide, oxidized glutathione and nitrotyrosine (see, *e.g.*, Pereira (1998) *Braz. J. Med. Biol. Res.* 31:827-833; Tsai (1998) *Gut* 42:850-855; De La Cruz (1998) *Brain Res.* 800:136-144; Paolisso (1998) *J. Am. Geriatr. Soc.* 46:833-838; Parthiban (1995) *Cell Biol. Int.* 19:987-93).

The term "pharmaceutical composition" refers to a composition suitable for pharmaceutical use in a subject. The pharmaceutical compositions of this invention are formulations that comprise a pharmacologically effective amount of a probucol ester and a pharmaceutically acceptable carrier. A "pharmacologically effective amount (or dose)" is the amount of compound (*e.g.*, a pharmaceutical composition comprising a probucol ester of the invention) administered to the individual to prophylactically or therapeutically effectively treat an individual for an injury, disease, infection, drug side effect, or other condition; *e.g.*, inhibition or prevention of oxidation (such as, *e.g.*, lipid oxidation), treatment of peripheral neuropathies, central nervous system diseases, neurovascular disorders associated with diabetes, opthalmic disorders, hearing disorders, pulmonary disorders, viral infections, cardiovascular diseases, hypercholesteremia, pre-eclampsia and eclampsia, male and female infertility, endometriosis, and the like. The skilled artisan can routinely determine the pharmacologically effective dose for a given situation, as discussed in detail, below.

The term "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use including, *e.g.*, metal salts (*e.g.*, sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines. Preferred embodiments of the invention include probucol ester compounds in the form of pharmaceutically acceptable salts.

The term "probucol" refers to a composition also known by the chemical designations " [4,4'-(isopropylidenedithio)bis(2,6-di-tert-butylphenol)]" and "bis(3,5-di-tert-butyl-4-hydroxyphenyl) acetone mercaptole." Probucol has been commercially available as a drug under the trademark Lorelco® (Marion Merrell Dow Pharmaceuticals, Inc., Kansas City, MO).

The term "prophylactic device" refers to products of manufacture used in the prevention of transmittal of sexually transmitted disease or as a contraceptive device. A prophylactic device can be a male or female condom or other barrier device, a spermicide, a contraceptive foam, and the like.

The term "subject" refers to treatment of an animal, such as a mammal, including a human. Non-human animals subject to treatment include, for example, fish, birds, and mammals such as cows, sheep, pigs, horses, dogs and cats.

The term "tablet" is used in its common context, and refers to a solid composition made by compressing and/or molding a mixture of compositions in a form convenient for swallowing or application to a body cavity.

The term "applied topically" refers to any non-internal application or adminstration of a compound, *i.e.*, any *ex vivo* application or administration.

The term "treatment" refers to prophylactic and therapeutic treatment administered to a subject. Prophylactic treatment includes administering treatment to an individual who does not have a disease or condition, or does not exhibit signs of a disease or condition, or exhibits only early signs of a disease or condition for the purpose of decreasing or abrogating the risk of acquiring the disease or condition or decreasing or abrogating a symptom or a pathologic condition arising from or related to the disease or pathology. Such an early sign can be, *e.g.*, elevated serum cholesterol. Thus, administration of a probucol ester of the invention has a prophylactic or treatment effect in inhibiting, ameliorating or preventing oxidative tissue damage in a person who is exposed to an oxidant, has experienced a trauma, or who is considered a high risk for developing a disease or condition with an inflammatory component, has a prophylactic or treatment effect in inhibiting, ameliorating or preventing oxidative tissue damage. Therapeutic treatment includes administering treatment to an individual who exhibits signs of pathology or injury due to disease, trauma, or other condition, or is considered at risk for developing or incurring such a condition (disease or injury), for the purpose of preventing, diminishing, ameliorating or eliminating any injury, tissue or chemical damage or pathology.

Probucol Phosphate Esters

The invention provides for novel phosphoric acid derivatives of probucol. The probucol esters of the invention are significantly more water-soluble than probucol, making them extremely useful as improved, probucol-based pharmaceuticals. Thus, the probucol esters of the invention are useful as antioxidants and as pro-drugs of probucol.

Preferred probucol ester embodiments are probucol monophosphate and probucol diphosphate.

The water-insolubility of probucol interferes with the predictability of its absorption by the body and makes it very difficult to administer in aqueous vehicles. The polar probucol esters of the invention, being significantly more soluble in water, clinically significantly enhance systemic absorption. The probucol esters, once absorbed, can be hydrolyzed to additional pharmacologically active forms; *e.g.*, probucol diphosphate is hydrolyzed to probucol monophosphate and probucol, and the monophosphate form is hydrolyzed to probucol. Once the probucol ester has been hydrolyzed to probucol, it re-partitions from an aqueous medium to a biological lipophilic medium, such as a cell membrane lipid bilayer or lipoprotein. Thus, the probucol esters of the invention can act as probucol pro-drugs. This invention also provides a variety of probucol-based pharmaceuticals which can be administered in aqueous vehicles, *e.g.*, intravenously, intraperitoneally, intrathecally, orally, as an inhalant or spray, and the like.

The only readily chemically reactive functionalities in probucol are its two phenolic hydroxyl groups. Since both phenolic hydroxyl groups are flanked by bulky tertiary-butyl substituents, they are blocked and therefore highly unaccessible. Adding a functional group to the phenolic oxygens is thus very difficult.

Development of a phosphate ester of probucol with improved solubility and stability properties has presented many difficulties. In fact, dozens of attempts to react probucol with highly active phosphorylation reagents, such as phosphorous oxychloride and phosphoric anhydride, under a variety of conditions, *e.g.*, tertiary amine catalysis, activating solvents, high temperature, failed to produce any detectable yield of probucol phosphate esters.

The invention includes the discovery that phosphorylation of probucol can be achieved if the phenolic hydroxyls are fully ionized prior to reaction with phosphorous oxychloride. Full ionization of the phenolic hydroxyls of probucol is achieved by reaction with alkyl-lithiums. Oxygen is carefully excluded from the reaction mixture in order to avoid rapid oxidative degradation of the lithiated probucol. In this manner, mixtures of phosphate esters of probucol are produced. The principal products of the reaction are the monophosphate and diphosphate probucol esters of the invention. Separation and purification of probucol phosphate esters is accomplished by standard techniques.

Another significant improvement of phosphate esters of probucol over probucol is their relative stability in an aqueous environment. They are not excessively reactive to hydrolysis. From a pharmacologic perspective, this is an ideal combination of water solubility, which allows the drug to be systemically absorbed in the body, and relatively slow rate of hydrolysis, providing an additional pro-drug advantage. Probucol phosphate esters prove to be quite stable to hydrolysis under both strongly acidic conditions, as low as a pH of about 1, and strongly alkaline conditions, as high as a pH of about 14. For example, probucol diphosphate has an estimated half-life of three weeks at pH 1, hydrolyzing to probucol monophosphate. Under these conditions, no significant hydrolysis to the unphosphorylated probucol occurs. At pH 14, probucol diphosphate does not measurably hydrolyze (see Example 6, below).

However, a slow, spontaneous hydrolysis of the phosphate ester moieties of probucol mono- and di-phosphates occurs at about pH 4 to about pH 7.4. Probucol diphosphate has an estimated half-life of 15 hours at a pH of between 4 and 6, hydrolyzing to probucol monophosphate; and an estimated half-life of 2 weeks at a pH of between 4 and 6 for complete ester hydrolysis to probucol. At pH 7.4, the estimated half-life of probucol diphosphate hydrolyzing to its monophosphate and unphosphorylated forms is 30 hours and 4 weeks, respectively (see Example 6).

Thus, in aqueous solutions such as those seen under physiologic conditions probucol diphosphate ester is converted cleanly and sequentially to probucol monophosphate ester, and thence probucol. Importantly, this hydrolytic conversion of probucol phosphate esters to probucol occurs at a pharmacologically significant rate *in vivo* (which is related to the concentration of probucol that is needed to achieve a therapeutic effect). Thus, the probucol ester pharmaceutical compositions of the invention, in addition to acting as anti-oxidants in the phosphorylated form (in an aqueous medium), also function as efficacious pro-drugs of probucol (which partitions into a lipophilic medium). The utility of the probucol esters of the invention are not limited by any particular mechanism of action, such as their ability to hydrolyze to probucol *in vivo*.

Hydrolysis of probucol esters is accelerated in the presence of enzymes, such as phosphatases. Under physiologic conditions, acid phosphatase is one of the more effective enzymes catalyzing this hydrolysis. *In vitro* enzymatic hydrolysis of probucol diphosphate appears to stop or to slow down significantly at the monophosphate ester, which may be a consequence of the formation of micelles of the monophosphate ester (see Example 7, below). The probucol monophosphate is partially soluble in water and forms

micelles (essentially, all of the monophosphate probucol is found in micellar form, above a minimum concentration). Thus, the monophosphate probucol of the invention in pharmaceutical formulations is typically found in micellar form. Probucol diphosphate ester is fully soluble in aqueous buffers and does not form micelles.

Taking advantage of these different rates of phosphate ester group hydrolysis and different relative water solubilities, in different embodiments, formulations of the invention can include only one particular probucol ester specie, such as only diphosphate probucol, only monophosphate probucol, only diprobucol phosphate, or varying amounts of any probucol ester of the invention. In other embodiments of the invention, probucol is included in a formulation with one of the novel probucol esters of the invention.

The monophosphate and diphosphate esters of probucol are demonstrated by the Examples set forth herein as effective antioxidant pharmaceuticals.

Probucol Esters as Anti-Oxidant Pharmaceutical Agents

The probucol ester compounds of this invention are useful as pharmaceutical agents to prevent or inhibit the oxidation of biological molecules and materials. The invention provides a method for inhibiting oxidation in a biological material, *e.g.*, a cell, a tissue, or an organism, comprising administering a probucol ester of the invention in an amount effective to inhibit the oxidation. Such inhibition can be extremely beneficial, because oxidation can be a source of serious damage to or premature loss (as through clearance or inactivation) of bioorganic molecules, including nucleic acids, proteins, lipids and carbohydrates. The source of oxidants can be external to an organism, *e.g.*, from the environment, or internal, *e.g.*, as a result of the natural or pathogenic production of free radicals or other oxidizing agents.

Reactive oxygen species, including free radicals, are produced through a number of naturally occurring biochemical reactions, often as a consequence of aerobic metabolism. A system including antioxidant enzymes and scavenger molecules serves to inhibit oxidation of biological molecules by these oxidants (*e.g.*, reactive oxygen species and free radicals), thus protecting membrane lipids, proteins, carbohydrates, and nucleic acids (e.g., DNA and RNA). An imbalance between production of reactive oxygen species and antioxidant protection results in "oxidative stress." Oxidative stress in tissues can be provoked by an augmented metabolic rate, a decrease in the antioxidant capacity, or many other factors. A natural source of oxidative stress arises from the fact that molecular oxygen has two unpaired electrons in its outer valence shells. The univalent reduction of molecular

oxygen generates a reactive radical called superoxide. The reductive environment of the cellular milieu provides ample opportunities for oxygen to undergo univalent reduction. Thus superoxide and other reactive molecules that can be produced from it, such as hydrogen peroxide and the extremely reactive hydroxyl radical, are common products of life in an aerobic environment, and these agents appear to be responsible for oxygen toxicity. Oxidative damage to biological material can also be caused by various enzymatic reactions related to the mitochondria (the stepwise reduction of oxygen by the mitochondrial electron-transport chain to produce water), the inflammatory process (discussed below), and the like. The oxidative damage can be in part mediated by an enzyme such as, *e.g.*, myeloperoxidase, superoxide dismutase or nitric oxide synthase. The probucol esters of the invention can be used to prevent or ameliorate such oxidative damage.

Exposure to environmental insults, such as tobacco smoke, pesticides, pollutants, and the like, can also cause oxidative stress. Inflammation-mediated pathology or cancer is commonly the result of such exposure. For example, exposure of lung alveolar macrophages to air pollution particulates causes substantial intracellular oxidant stress (Goldsmith (1997) *Environ. Health Perspect.* 105 Suppl 5:1191-1195). Cigarette smoke can create oxidative stress, primarily through chemical modification of glutathione as a major damage mechanism (Maranzana (1998) *Arch. Biochem. Biophys.* 350:169-182). Oxidative stress as a causative agent in cancer is discussed below. The probucol esters of the invention can be used to prevent or ameliorate such environmental damage.

The probucol esters of the invention, as effective anti-oxidants *in vivo*, can treat or prevent oxidative stress and the damage directly or indirected induced by this stress (*e.g.*, caused by inflammatory components of an immune response) of a wide variety of injuries, degenerative processes, diseases and syndromes, and other conditions. These include, but are not limited to, *e.g.*, mutagenesis, cell transformation and cancer, atherosclerosis, arteriosclerosis, myocardial infarction ("heart attack"), cerebrovascular accidents ("strokes"), and ischemia/reperfusion injury and restenosis, pre-eclampsia and eclampsia, neurovascular disease associated with diabetes, chronic renal failure patients undergoing peritoneal dialysis or hemodialysis, chronic inflammatory diseases, such as rheumatoid arthritis, psoriatic arthritis, osteoarthritis, lupus erythematosus and psoriatic arthritis, acute inflammatory problems, such as wound healing, photo-oxidative stresses to the eye (such as cataract) and to the skin (as in melanoma and other skin cancers), hearing loss (such as noise-induced hearing loss), central-nervous-system disorders, such as certain forms of familial amyotrophic lateral sclerosis, certain glutathione peroxidase-linked

adolescent seizures, Parkinson's disease and Alzheimer's dementia, and a wide variety of age-related disorders, including factors underlying the aging process itself, and fatigue of skeletal muscles. Oxidative stress is also implicated in the pathological processes resulting from CNS trauma, including spinal cord injury. Some of these oxidation-linked diseases or disorders can be exacerbated, perhaps even initiated, by numerous environmental pro-oxidants and/or pro-oxidant drugs and foods. Oxidative stress is also associated with infections, *e.g.*, infections by mammalian viruses, particularly those with lipid membranes. The probucol ester compounds of this invention are useful as anti-oxidative pharmaceutical agents to prevent or inhibit the oxidative stress and damage caused by or associated with all of these conditions.

Probucol Esters as Anti-Inflammatory Pharmaceutical Agents

The probucol esters of the invention, as anti-oxidant compounds, are useful in the treatment, amelioration and prevention of conditions having an inflammatory component. The invention provides a method for the treatment of a condition with an inflammatory component in a subject, comprising administering to the subject a probucol ester of the invention in an amount effective to treat the condition. Many, if not most, diseases and injuries involve an inflammatory component which contributes to the pathogenesis or damage caused by the condition. The probucol esters of the invention, as anti-oxidants, are useful as pharmaceuticals in the treatment and prevention of deleterious inflammatory insult(s). While the invention is not limited to or by a particular theory of operation, the compositions of the invention decrease the amount of damaging oxidating compositions generated by the inflammatory response.

Inflammation is characterized at the cellular level by the production of inflammatory mediators, such as cytokines and eicosanoids, *e.g.*, prostaglandins, prostacyclins, thromboxanes and leukotrienes. At the tissue level, inflammation is characterized by invasion of leukocytes, especially neutrophils, macrophages and lymphocytes. For example, neutrophils, when stimulated, generate reactive oxygen species, including myeloperoxidase-derived hypochlorous acid, or HOCl. Neutrophils can oxidize nearly all of the amino acids commonly found in plasma to a corresponding family of aldehydes in high yield. The reaction is mediated by hypochlorous acid (HOCl), the major oxidant generated by the myeloperoxidase-H2O2-Cl- system of phagocytes.

The production of prostaglandins, prostacyclins and thromboxanes typically involve an oxidation step that is cyclooxygenase dependent. For example, protein nitration is a final product of the production of highly reactive nitrogen oxide intermediates (*e.g.*, peroxynitrite) formed in reactions between nitric oxide (NO) and oxygen-derived species such as superoxide. The enzyme prostaglandin H synthase-2 forms one or more tyrosyl radicals during its enzymatic catalysis of prostaglandin formation. Arachidonic acid, an unsaturated fatty acid constituent of the phospholipid domain of cell membranes, is released via mobilization of phospholipases, is subject to free radical attack and peroxidation, generating F2-isoprostanes.

Disease and conditions with inflammatory components which can be treated or prevented using the probucol ester compositions of the invention include, but are not limited to, *e.g.*, those listed above as associated with oxidative stress.

Probucol Esters as Anti-Viral Pharmaceutical Agents

The anti-oxidant probucol esters of the invention are effective anti-viral and anti-retroviral agents. The invention provides a method for the treatment of a viral infection in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the viral infection. While the invention is not limited to or by a particular theory of operation, the anti-viral and anti-retroviral efficacy may be through an effect on viral membrane fluidity, or the anti-oxidant's ability to prevent lipid peroxidation, or their preferential solubility in viral lipid membranes, or a combination of these or other mechanisms. Accordingly, anti-oxidant probucol esters are effective as antiviral agents against mammalian viruses, particularly those with lipid membranes, such as, *e.g.*, herpes viruses, such as herpes simplex viruses, *e.g.*, herpes simplex virus 1 (HSV1) or herpes simplex virus (HSV2), and human herpes virus 6 (HHV6), HHV7, and HHV8); cytomegalovirus (CMV); Epstein Barr virus (EBV); varicella zoster virus (VZV); paramyxovirus; vaccinia virus (Baek (1997) *J. Biol. Chem.* 272:32042-32049); retroviruses, such as HIV-1 (Munoz-Barroso (1998) *J. Cell. Biol.* 140:315-323), HIV-2, HTLV-1, HTLV-2; Ebola virus (Ruiz-Arguello (1998) *J. Virol.* 72:1775-1781); and, Marek's disease virus.

The anti-viral and anti-retroviral efficacy of the anti-oxidant probucol esters of the invention can also be through their ability to ameliorate oxidative stress, *i.e.*, to decrease the levels of reactive oxygen species in an individual. Increased levels of reactive oxygen species are implicated in the regulation of mammalian transcription factors, including nuclear factor NFκB and AP1. NFκB and AP1 are influenced by cellular redox

status, and have been implicated in the transcriptional regulation of a wide variety of genes involved in the cellular inflammatory process and tissue destruction. NFκB is believed to play a central role in the inflammatory cellular process (see, *e.g.*, Winyard (1997) "Antioxidants, redox-regulated transcription factors, and inflammation," *Adv Pharmacol.* 38:403-421). Furthermore, retroviral (*e.g.*, HIV) activation via NFκB is a direct consequence of increased levels of reactive oxygen species. In AIDS, expression of HIV in the infected lymphocyte is NFκB dependent (see, *e.g.*, Kotler (1998) "Antioxidant therapy and HIV infection," *Am. J. Clin. Nutr.* 67:7-9, Allard (1998) *Am. J. Clin. Nutr.* 67:143-147). Accordingly, the anti-oxidant probucol esters of the invention, in decreasing the severity of or inhibiting oxidative stress and lowering levels of NFκB, are effective anti-retroviral pharmaceutical reagents. The probucol esters can be used alone or in combination with other anti-HIV pharmaceuticals for patients with AIDS.

Programmed cell death (apoptosis) of T-lymphocytes observed in HIV infected individuals has been linked to oxidative stress. Reactive oxygen species induce apoptosis, which contributes to the cell loss during progression of HIV-1 infection. Reactive oxygen species-induced apoptosis is significantly enhanced in HIV-infected subjects even in the very early stages after infection. Moreover, reactive oxygen species-mediated apoptosis is not restricted to a particular lymphocyte subset (Dobmeyer (1997) *Free Radic. Biol. Med.* 22:775-785). In view of the diminished oxidative resistance of HIV-infected individuals, administration of the anti-oxidant probucol esters of the invention, by decreasing the level of oxidative stress, are effective in decreasing the amount of reactive oxygen species-mediated apoptosis contributing to the deletion of lymphocytes and to the pathogenesis of AIDS.

The anti-oxidant probucol esters of the invention can also be used in the *ex vivo* treatment of cells infected with retroviruses. HIV infected CD4+ helper T cells can be removed from the patient, treated with the probucol esters of the invention *ex vivo*, and reinfused. Such *ex vivo* manipulation of lymphocytes has been done, for example, with autologous CD8+ cytotoxic T cells enriched for HIV-specific cytotoxicity targeted against a diversity of HIV epitopes (Lieberman (1997) *Blood* 90:2196-2206; Wilson (1995) *J. Infect. Dis.* 172:88-96).

Probucol has been shown to have anti-viral activity against adult patients with HIV-1, as described in Hendler, U.S. Patent No. 4,985,465. With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the treatment of viral and retroviral infection and disease.

Probucol Esters as Pharmaceutical Agents to Treat Reperfusion Injury

The anti-oxidant probucol esters of the invention are effective pharmaceutical agents in the treatment and prevention of reperfusion injury that is the result of surgery (*e.g.*, cardiac surgery, such as bypass surgery, or organ transplantation), injury, infarctions (*e.g.*, heart attacks or cerebral ischemia/stroke), fibrinolytic procedures (*e.g.*, streptokinase) and the like. The invention provides a method for the treatment of reperfusion injury in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the reperfusion injury.

While the invention is not limited to or by a particular theory of operation, the compositions of the invention can be pharmacologically efficacious by decreasing the amount of oxidatively damaged tissue, especially, vascular muscle tissue. Oxygen free radicals play a major role in ischemic injury, particularly when followed by reperfusion. Involvement of oxygen free radicals in ischemia-/reperfusion injury is based on measurements of increased products of lipid peroxidation after organ ischemia and restoration of blood flow during surgical operations and reperfusion of organ transplants. For example, ischemia/reperfusion of human skeletal muscle involves both xanthine oxidase-dependent oxygen free radicals and cyclooxygenase metabolites. These pathways activate circulating neutrophils, which inflict local and remote endothelial injury. Furthermore, excitotoxic or ischemic conditions excessively activate the neuronal isoform of nitric oxide synthase (nNOS), resulting in concentrations of nitric oxide (NO) that are toxic to surrounding neurons. The immunologic isoform of NOS (iNOS), not normally present in healthy tissue, is induced shortly after ischemia and contributes to secondary late-phase damage.

In ischemia, delayed post-ischemic hypoperfusion develops after a preceding phase of post-ischemic hyperemia and is characterized by increased vasotonus. Hypo-perfusion is associated with dysfunction in metabolism and blood flow caused, in part, by the generation of free radicals and possibly down-regulation of endothelial nitric oxide synthase. Current treatment of post-ischemic hypoperfusion, which includes neutrophil elimination

and free radical scavengers, is still unsatisfactory. Use of the probucol esters of the invention can be an important adjunct in the treatment of ischemic cell injury, especially in the case of cerebral ischemia, organ transplantation, peripheral artery occlusion, and heart surgery. Probucol esters can also be used adjunctively with reperfusion protocols, as in the treatment of myocardial infarction with balloon angioplasty or in the treatment of stroke with fibrinolytic agents. Prophylactic use of probucol esters before a reperfusion procedure can abrogate or ameliorate the severity of reperfusion injury.

The inflammatory response to cardiopulmonary bypass and reperfusion procedures is the product of a complex interplay of humoral and cellular components. Contact activation cascades, the complement system, and cytokines comprise the humoral elements and interact in such a way as to propagate their own cascades and to activate the cellular elements. Cellular components include neutrophils and endothelial cells, which become involved after their activation by humoral mediators. Neutrophil-endothelial cell adherence is the initial step of the cellular inflammatory response. It is promoted by the expression of specific adhesion molecules on the surfaces of both of these cells. This leads to the emigration of neutrophils into the extravascular space where they release toxins, including oxidants, that damage surrounding tissues. The resulting organ dysfunction produces the clinical picture referred to as the "postperfusion syndrome." Strategies to attenuate this response, including the administration of corticosteroids, aprotinin, and anticytokine monoclonal antibodies, are inadequate to prevent or significantly ameliorate resultant tissue damage. The probucol esters of the invention can be used as clinically efficacious agents to better prevent such injury.

Probucol has been shown to have a protective effect on myocardium with respect to ischemia-reperfusion arrhythmia in an isolated rat heart (Tada (1992) *Can. J. Cardiol.* 8:975-980). In another study, the effects of short-term (7 days) or long-term (24 weeks) administration of 1% probucol on the size of infarcts resulting from 30 minutes of coronary occlusion followed by reperfusion (for 48 hours) in hyperlipidemic rabbits

demonstrated that infarction lesions in untreated rabbits were significantly larger than those in the rabbits receiving the long-term treatment with probucol. Long-term probucol treatment also significantly reduced myeloperoxidase activity in both ischemic and nonischemic myocardium (Hoshida (1997) *Arterioscler. Thromb. Vasc. Biol.* 17:2801-2807). With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the treatment and prevention of reperfusion injuries.

Probucol Esters as Pharmaceutical Agents to Treat Restenosis

Restenosis after coronary angioplasty (*e.g.*, percutaneous transluminal coronary angioplasty or PTCA) is a major limitation of an otherwise highly effective and safe procedure for the treatment of atherosclerotic coronary artery disease. The invention provides a method for the prevention and treatment of restenosis in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to prevent and treat the restenosis.

Although the advent of coronary stenting has reduced restenosis rates for selected patients, chronic restenosis of dilated lesions remains a serious and frequent problem, occurring in 30% to 50% of cases. With a peak incidence between 1 and 4 months after the angioplasty procedure, restenosis has been thought to reflect an exaggerated healing response to balloon injury. In such an injury, platelets and other cells secrete mitogens that induce smooth muscle cells from the arterial media to migrate to and proliferate in the intima. This results in narrowing of the coronary luminal vessels, invoking ischemic symptoms. Despite numerous clinical trials, no effective pharmacologic therapy has been found to prevent the occurrence of this post-procedural complication. The probucol esters of this invention can be used as clinically efficacious agents to better prevent and ameliorate this post-surgical complication.

Neointimal thickening, also referred to as neointimal hyperplasia, occurs in response to arterial injury and coronary (balloon) angioplasty. This hyperplastic process involves different steps which include smooth muscle cell activation, proliferation, and migration, and the production of extracellular matrix. The factors which control neointimal thickening include growth factors, hormonal factors, and mechanical factors. Neointimal formation and arterial wall remodeling are pivotal causes of luminal narrowing in both atherogenesis and restenosis. Arterial remodeling refers to a series of dynamic structural

changes - such as lumen loss and restenosis - that arteries may undergo in response to various stimuli, including changes in blood flow and pressure, and acute injury.

Probucol reduces lumen loss and restenosis rate after balloon angioplasty in small coronary arteries (Rodes (1998) *Circulation* 97:429-436; Edelman (1998) *Circulation* 97:416-420; Lisi (1997) *N. Engl. J. Med.* 337:1919; Jay (1997) *N. Engl. J. Med.* 337:1918-1919; Sirtori (1997) *N. Engl. J. Med.* 337:1918; Yokoi (1997) *J. Am. Coll. Cardiol.* 30:855-862; Libby (1997) *N. Engl. J. Med.* 337:418-419; Tardif (1997) *N. Engl. J. Med.* 337:365-372). For example, in one study, one month before angioplasty, patients were randomly assigned to one of four treatments: placebo, probucol (500 mg), multivitamins, or probucol plus multivitamins twice daily. The treatment was maintained until follow-up angiography was performed at 6 months. Probucol was found to significantly reduce loss of luminal diameter and restenosis (Rodes (1998) *supra*). With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the treatment and prevention of restenosis. Such treatment would not only reduce morbidity and mortality but would also significantly reduce medical expenses.

Probucol Esters as Pharmaceutical Agents to Treat Hypercholesterolemia and Hyperlipidemia

The anti-oxidant probucol esters of the invention are effective pharmaceutical agents in the treatment and control of hypercholesterolemia and hyperlipidemia. The invention provides a method for the treatment of hypercholesterolemia or lipidemia in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the hypercholesterolemia or lipidemia. While the invention is not limited to or by a particular theory of operation, the compositions of the invention can be pharmacologically efficacious by various mechanisms, including inhibiting the oxidation of low density lipoproteins (LDLs).

Probucol was found to significantly reduce serum levels of cholesterol and lipids. For example, long-term probucol treatment significantly reduced the surface area of atherosclerotic plaque lesions in the aorta in a rabbit model (Hoshida (1997) *supra*). In one human clinical study, the therapeutic effect of probucol on hypercholesterolemia in cyclosporine a (CyA)-treated renal transplant patients was investigated. Twelve post-transplantation patients with high serum total cholesterol (CHL) (250 mg/dL or greater) were treated with probucol, 250 mg twice daily for 3 months. After treatment with probucol, serum t-CHL, LDL-CHL, high-density lipoprotein (HDL)-CHL, phospholipids (PL), LDL-PL, and apoprotein AI were significantly decreased, and CHL-ester significantly increased compared with the pretreatment levels. Probucol caused a decrease in HDL-CHL and acted anti-atherogenically by modulating HDL metabolism and stimulating reverse transfer of CHL from peripheral tissue (Okubo (1998) *Am. J. Kidney Dis.* 31:356-359). In another clinical study, probucol was administered to patients with hypercholesterolemia as a single drug at a dose of 0.5 g tablets twice a day for at least 6 months; 71% patients displayed a reduction of LDL-CHL; 90% had a reduction of HDL-CHL (Baldassarre (1997) *J. Cardiovasc. Pharmacol.* 30:784-789). In another study where probucol was administered to hypercholesterolemic patients, probucol-treated patients had 15% lower total CHL and 35% lower HDL-CHL compared with controls. LDL from probucol treated individuals was more resistant to oxidation by Cu^{2+} and showed a 13 times lower formation of lipid peroxides; a 97% reduction in macrophage degradation and close to 90% less decrease in LDL receptor binding following oxidation as compared with controls was also seen (Regnstrom (1996) *Atherosclerosis* 125:217-229). With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the treatment and prevention of hypercholesterolemia and hyperlipidemia.

Probucol Esters as Pharmaceutical Agents to Treat Atherosclerotic Vascular Disease

The probucol esters of the invention, as anti-oxidants, are useful as pharmaceuticals in the treatment and prevention of atherosclerotic vascular disease. The invention provides a method for the treatment of an atherosclerotic cardiovascular disease in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the cardiovascular disease. While the invention is not limited to or

by a particular theory of operation, the compositions of the invention can be pharmacologically effective by decreasing the amount of oxidatively damaged LDLs that are believed to play a critical role in the pathogenesis of atherosclerotic vascular disease. The correlation between the presence of lipid-laden macrophage foam cells, lipid oxidation, alpha-tocopherol depletion, ceroid accumulation, and macrophage death in advanced lesions, strongly supports a role for oxidative damage in atherosclerosis.

Macrophages exposed to increased levels of LDL generate an intracellular oxidative response. Macrophage mediated oxidation of LDL to ox-LDL is cytotoxic to macrophages due to oxidative damage of lysosomal membranes, with ensuing destabilization and leakage to the cytosol of lysosomal contents, such as hydrolytic enzymes, causing degeneration into foam cells and apoptosis. Reactive oxygen species-mediated mitochondrial-dependent pathways also contribute to the apoptotic process by causing nuclear damage. This nuclear damage in the form of DNA strand breakage is mediated as a normal consequence of this increased oxidative stress, which is a signal to increase p53 protein levels and apoptosis (programmed cell death). This macrophage cell death is believed to contribute to the formation of atherosclerotic plaques.

Mass spectrometric analysis of protein oxidation products isolated from atherosclerotic tissue also implicate tyrosyl radical, reactive nitrogen intermediates and hypochlorous acid in LDL oxidation and lesion formation *in vivo*. Hypochlorous acid is generated by the phagocytic enzyme myeloperoxidase, which can also generate tyrosyl radical and reactive nitrogen intermediates. Cellular lipoxygenases also play a role at certain stages. With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the prevention of or decrease in the rate of formation of tyrosyl radicals, reactive nitrogen intermediates, hypochlorous acid, macrophage foam cell formation, and atherosclerotic plaques.

Probucol Esters as Pharmaceutical Agents to Treat Central and Peripheral Nervous System Disorders and Trauma

The invention provides a method for the treatment of a central or a peripheral neuropathy with an inflammatory component in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the neuropathy. The probucol esters of this invention are particularly useful in inhibiting oxidation and its resultant damage in disorders and trauma of the central and peripheral nervous systems that

involve an inflammatory component. This results both from their anti-oxidant properties and because, once introduced into the system, their ability to rapidly equilibrate in various anatomical compartments. For example, while probucol is thought to have little or no access to the central nervous system (CNS), it is believed that the probucol esters of the invention may have enhanced access into the central nervous system. Furthermore, probucol has been observed to mitigate the symptoms of severe painful peripheral neuropathy in patients with advanced AIDS (Nagourney, et al., (1994) abstract, International AIDS meeting).

Oxidative stress has been implicated in both normal aging and in various neurodegenerative disorders and may be a common mechanism underlying various forms of neuronal cell death including necrosis, apoptosis, and excitotoxicity. Peripheral nervous system pathologic conditions that can be treated with the probucol esters of the invention include, *e.g.*, neurodegenerative diseases, trauma, and diabetes (increased oxygen free radical activity, coupled with reduced protection against oxidative stress, plays a role in the etiology of neurovascular abnormalities in diabetes mellitus, Cameron (1994) *Diabetologia* 37:449-459; see also, Van Dam (1997) *Neuroscience Research Communications* 21 (1): 41-48). Central nervous system pathologic conditions that can be treated or ameliorated with the probucol esters of the invention include, *e.g.*, Friedrich's disease, Parkinson's disease, Alzheimer's disease, Huntington's disease, Pick's disease (a form of frontotemporal dementia), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS).

The degeneration of neurons in Parkinson's disease (PD) involves oxidative stress as manifested by increased lipid peroxidation and oxidative DNA damage in various areas of brain, including the substantia nigra, caudate nucleus, and putamen (Alam (1997) *J. Neurochem.* 69:1326-1329). The oxidative damage in PD is caused, in part, by excessive formation of reactive oxygen species and loss of systems involved with scavenging or preventing the formation of such radicals from hydrogen peroxide generated as a consequence of dopamine oxidation (through autoxidation and deamination) (Youdim (1994) *Life Sci.* 55:2077-2082). Oxidative stress-mediated neuronal loss is in part mediated by a decline in the antioxidant molecule glutathione (GSH). GSH plays multiple roles in the nervous system including free radical scavenger, redox modulator of ionotropic receptor activity, and possibly, as neurotransmitter. GSH depletion can enhance oxidative stress and increase levels of excitotoxic molecules, thus initiating cell death in distinct neuronal populations. Oxidative stress and diminished GSH is associated with the pathogenesis of amyotrophic lateral sclerosis (ALS, or "Lou Gehrig's disease"), Parkinson's disease, and Alzheimer's disease (Bains (1997) *Brain Res. Rev.* 25:335-358).

Free radical and oxidative stress induced injury also mediate glutamate neurotoxicity causative for several neurodegenerative disorders including, cerebrovascular disease and accidents ("stroke"), seizure disorders, epilepsy, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS). Activation of the N-methyl-D-aspartate (NMDA) receptor subtype of glutamate receptors results in the influx of calcium which binds calmodulin and activates neuronal nitric oxide synthase (nNOS), to convent L-arginine to citrulline and nitric oxide (NO). When generated in excess, NO can be neurotoxic; most of the neurotoxic actions of NO are mediated by peroxynitrite (ONOO-), the reaction product of NO and superoxide anion. In pathologic conditions, peroxynitrite and oxygen free radicals can be generated in excess of a cell antioxidant capacity resulting in severe damage to cellular constituents including proteins, carbohydrates, nucleic acids (e.g., RNA and DNA) and lipids. The inherent biochemical and physiological characteristics of the brain, including high lipid concentrations and energy requirements, make it particularly susceptible to free radical and oxidant mediated insult and responsive to anti-oxidant treatment.

Most traumas to the central nervous system, such as skull fracture and its resulting edema, brain hemorrhages, shearing lesions, subdural and epidural hematoma, and spinal cord injury (e.g., mechanical injury due to compression or flexion of the spinal cord), have at least one damaging inflammatory component.

Probucol has been found to be efficacious in treating and ameliorating neurodegenerative disease. For example, in one study, the efficacy of antioxidant agents, including probucol, in preventing toxicity caused by oxidative insults (iron, hydrogen peroxide, and tert-butyl hydroperoxide) and beta-amyloid peptides in cultured rat hippocampal neurons was investigated (beta-amyloid peptides mediate pathogenesis in Alzheimer's disease). Increasing basal levels of oxidative stress by pretreating cultures with subtoxic doses of iron significantly increased neuronal vulnerability to beta-amyloid. The ability of beta-amyloid to induce oxidative stress and the demonstration that oxidative stress potentiates beta-amyloid toxicity support the clinical use of the antioxidant probucol esters of the invention in the treatment and prevention of Alzheimer's disease.

With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the prevention and/or treatment of free radical and oxidative stress induced neurologic disease and injury, glutamate neurotoxicity, and disorders and trauma of the central and peripheral nervous system.

In the treatment of pathologic conditions of the nervous system, the compound preferably is administered parenterally, such as by intravenous injection or injection directly into the central nervous system (*e.g.*, intrathecal).

Probucol Esters as Pharmaceutical Agents to Treat Opthalmic Disorders

The anti-oxidant probucol esters of the invention are effective pharmaceutical agents in the treatment and control of opthalmic disorders, such as cataracts and macular degeneration. The invention provides a method for the treatment of an opthalmic disorder in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the opthalmic disorder. While the invention is not limited to or by a particular theory of operation, the compositions of the invention can be pharmacologically efficacious by inhibiting the oxidation of lipids, with resultant vessel atherosclerosis, in the vasculature of the eye.

For example, age-related macular degeneration is a vascular disorder that is a manifestation of the hemodynamic consequence of the process of lipoid infiltration, or atherosclerosis of the vessels of the eye. The resultant decrease in blood perfusion leads to the atrophic form of age-related macular degeneration (Friedman (1997) *Am. J. Ophthalmol.* 124:677-682). As discussed above, anti-oxidants are particularly effective in the prevention and treatment of atherosclerotic lesions.

Antioxidants have also been shown to be anti-cataract agents, protecting the lens from oxidative stress. In one study, peroxidase (an antioxidant enzyme) was capable of protecting cultured rat lenses from photochemical stress mediated by hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl (OH^-) (Spector (1997) *Exp. Eye Res.* 65:457-47). Thus, with their properties of superior stability and water solubility, as anti-oxidants, the probucol esters of the invention can be extremely efficacious, preferred reagents for the treatment of macular degeneration, cataracts, and opthalmic disorders in general.

Probucol Esters as Pharmaceutical Agents to Treat Hearing Loss

The probucol esters of this invention, functioning as anti-oxidants, are useful in the treatment of hearing loss. Oxidative stress and reactive oxygen species have been implicated in hearing loss resulting from noise-induced coclear damage (see, e.g., Prasher (1998) Lancet 352:1240-1242).

The present invention provides a method for the treatment of hearing loss. Antioxidants administered, *e.g.*, intraperitoneally, orally or by instillation through catheters into the inner ear have been found to ameliorate noise-induced hearing loss. Thus, with their properties of superior stability and water solubility, as anti-oxidants, the probucol esters of the invention can be extremely efficacious and preferred reagents for the treatment of hearing loss.

Probucol Esters as Adjunctive Anti-Cancer Pharmaceutical Agents

The probucol esters of this invention, functioning as anti-oxidants, are useful in the treatment of cancer. The invention provides a method for the treatment of a drug-induced oxidative stress in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the drug-induced oxidative stress. Co-administration of anti-oxidants improves the outcome of chemotherapy in subjects with cancer (Kastan (1997) *Nature Medicine* 11:192-1195). Inflammation (caused by the cancer, cell death as a result of chemo- or radio-therapy or caused directly by the chemotherapeutic agent itself) typically is followed by the release of potentially damaging substances, such as reactive oxygen intermediates and lipid oxidation products from inflammatory cells. This inflammation has been linked to the potentiation of carcinogenesis. For example, murine macrophages when stimulated with phorbol esters induce 5,6- ring saturated thymine residues, a nucleic acid alteration of known oxidative origin, in co-cultivated mammalian cells. DNA can also be damaged by the major product of hydroxy-radical addition to tyrosine, 3,4-dihydroxyphenylalanine (DOPA). Protein-bound DOPA can produce oxidation products of DNA, including 8-oxo-7,8-dihydro-2'- deoxyguanosine and 5-hydroxy-2'-deoxycytidine (Morin (1998) *Biochem J.* 330:1059-1067). In addition, the teratogenicity of many xenobiotics is due at least in part upon their bioactivation by embryonic cytochromes P450, prostaglandin H synthase and lipoxygenases to electrophilic and/or free radical reactive intermediates that covalently bind to or oxidize cellular macromolecules such as DNA, protein and lipid, resulting in *in utero* death or teratogenesis (Wells (1997) *Mutat Res.* 396:65-78).

The invention also provides a method for the treatment of a cancer in a subject comprising administering to the subject a probucol ester in an amount effective to treat the cancer. Probucol has been shown to have a protective, anti-tumor effect. In one study, the effects of probucol on benzo[a]pyrene-induced pulmonary and forestomach tumorigenesis as well as induction of colonic aberrant crypt foci (ACF) in mice was

investigated. A probucol diet significantly reduced the numbers of large ACF, putative pre-neoplastic lesions of the colon mucosa (Zarkovic (1997) *Carcinogenesis* 16:2599-2601). In another study, the effects of dietary probucol on renal damage induced by a renal carcinogen, ferric nitrilotriacetate (Fe-NTA), in rats were investigated. The kidneys of rats fed a 1% probucol diet were protected from necrosis and lipid peroxidation induced by a single I.P. treatment with Fe-NTA solution at 5 mg Fe/kg body wt and were significantly resistant to a higher dose (Qin (1995) *Carcinogenesis* 16:2549-2552). Thus, administration of probucol ester, as an antioxidant, before an individual is exposed to a known carcinogen, during, or after exposure, can be effective in the treatment and prevention of cancer. With their properties of superior stability and water solubility, as pro-drugs for probucol, the probucol esters of the invention can be extremely efficacious, preferred reagents for the prevention and/or treatment of cancer. Probucol esters can be useful in the treatment of any cancer.

Administration of chemotherapeutic agents also frequently causes non-therapeutic inflammation as a side effect, thus limiting the duration and/or dosage of agent that can be given to the patient. For example, current knowledge about adriamycin cardiomyopathy (a side effect of adriamycin administration) indicates that the major cause of this condition is increased oxidative stress, although the drug's antitumor action in patients may involve other mechanisms (Singal (1997) *FASEB J.* 11:931-936). Thus, co-administration of probucol esters adjunctively with chemotherapeutic agents is beneficial in the treatment of cancer.

The probucol esters of the invention can be administered to subjects before, during and/or after chemotherapy. Preferred routes of administration include intravenous, parenteral and oral routes.

Probucol Esters as Pharmaceutical Agents to Treat Respiratory Disorders

The invention provides a method for the treatment of a pulmonary disorder in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the pulmonary disorder. Because they can be easily administered to the respiratory system via inhalation, the probucol esters of this invention are especially useful in the treatment of respiratory disorders having an inflammatory component or that result from exposure to (inhalation of) oxidizing agents or agents which cause tissue inflammation, such as ozone and high concentrations of oxygen itself. The probucol esters of the invention can inhibit oxidation and its subsequent damage that result in or accompany such respiratory disorders and exposures, such as, *e.g.*, acid aspiration, adult/infant respiratory distress syndrome, airway obstructive disease, asthma, bronchiolitis, bronchopulmonary dysplasia, cancer, chronic obstructive pulmonary disease ("COPD"), cystic fibrosis, emphysema, HIV-associated lung disease, idiopathic pulmonary fibrosis, immune-complex-mediated lung injury, ischemia-reperfusion injury, mineral dust pneumoconiosis, drug-induced lung disease, silo-filler's disease, and byssinosis (an occupational lung disease in textile mill workers exposed to the respirable dusts of cotton, hemp, and flax; see Bates (1995) *Exp. Lung Res.* 21:643-665), exposure to high concentrations of oxygen, and respiratory infections in general. Exposure to oxidizing agents such as dust (*e.g.*, silica, glass fiber, or coal dust) (Blackford (1997) *J. Toxicol. Environ. Health* 51:203-218), asbestos (asbestos generates free radicals, Rahman (1997) *Environ. Health Perspect.* 105 Suppl 5:1109-1112), ozone, air pollutants, nitric oxide, nitrogen dioxide, sulfur dioxide, tobacco smoke, diesel exhaust, or other combustion byproducts also result in inflammatory inflammation, and thus can be treated with the compounds of this invention.

In the treatment of respiratory conditions, the compound is preferably delivered by inhalation; however, it can be given orally and parenterally as well. The compound can be delivered as an aerosol, mist or powder.

Probucol Esters as Pharmaceutical Agents to Treat Arthritis

The invention provides a method for the treatment of an arthritis having an inflammatory component in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the arthritis. Rheumatoid arthritis, psoriatic arthritis, osteoarthritis, and the like, are pathologic conditions which have an inflammatory component. In one study, significantly increased lipid peroxidation, measured

as malondialdehyde (MDA), was demonstrated in the plasma of rheumatoid arthritis patients. The increased oxidant stress present in rheumatoid arthritis led to compensatory changes in the levels of some antioxidants, *e.g.*, glutathione and ceruloplasmin (Gambhir (1997) *Clin. Biochem.* 30:351-355). The synovitis of osteoarthritic results in the production of cytokines such as interleukin 1 alpha (IL-1 alpha), interleukin 1 beta (IL-1 beta), tumor necrosis factor alpha (TNF-alpha) that contribute to destructive pathogenic processes. Accordingly, probucol esters, as antioxidants, are useful in treating, ameliorating or preventing arthritis.

In treating arthritis, the compounds are preferably delivered orally, intravenously, parenterally, or transdermally for this purpose.

Probucol Esters as Pharmaceutical Agents to Treat Pre-eclampsia and Eclampsia

The invention provides a method for the treatment of pre-eclampsia and eclampsia in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the pre-eclampsia or eclampsia. Increased free-radical activity is involved in the pathogenesis of pre-eclampsia. Radical-scavenging antioxidants are consumed by an increased free-radical activity in pre-eclampsia. One study demonstrated that serum total antioxidant status levels in mild and severe pre-eclampsia and eclampsia were significantly lower than that of healthy pregnant women; the mean percent decreases amounted to 22% (mild pre-eclampsia), 40% (severe pre-eclampsia) and 59% (eclampsia), respectively, indicating the propensity of these patients to oxidative stress. (Shaarawy (1998) *Int. J. Gynaecol. Obstet.* 60:123-128). Accordingly, the compounds of this invention are useful in treating or ameliorating pre-eclampsia and eclampsia.

In treating pre-eclampsia and eclampsia, the compounds are preferably delivered orally, intravenously, or parenterally.

Probucol Esters as Pharmaceutical Agents to Treat Male and Female Infertility

The invention provides a method for the treatment of infertility due to increased oxidative stress in a subject comprising administering to the subject a probucol ester of the invention in an amount effective to treat the infertility. Excessive generation of reactive oxygen species by abnormal spermatozoa and by contaminating leukocytes has been identified as one of the few defined etiologies for male infertility. In one study, glutathione (an antioxidant) administered *in vivo* to patients who had infertility (whose etiology may have been in part due to excessive oxidative stress in the epididymis during

spermatogenesis) improved the function of ejaculated spermatozoa (Irvine (1996) *Rev. Reprod.*1:6-12). Thus, the probucol esters of the invention, as antioxidants, can be useful in the treatment of some types of male infertility.

Likewise, oxidative stress may be related to some types of female infertility; therefore, the probucol esters of this invention can be useful in these cases.

Probucol Esters as Pharmaceutical Agents to Treat Erectile Dysfunction

The invention provides a method for the treatment of erectile dysfunction. This condition is known to be mediated at least in part by abnormal reactive oxygen species metabolism. Antioxidant compounds play important roles in ameliorating reactions leading to erectile dysfunction. The compounds of the present invention are therefore useful in the treatment of erectile dysfunction.

Probucol Esters as Pharmaceutical Agents to Treat Alopecia

The invention provides a method for the treatment of alopecia (hair loss). This condition is known to be mediated at least in part by abnormal reactive oxygen species metabolism. Antioxidant compounds play important roles in ameliorating reactions leading to alopecia (see, *e.g.*, Wong (1995) *Biochim. Biophys. Acta* 1271:205-209). The compounds of the present invention are therefore useful in the treatment of alopecia.

Probucol Esters as Pharmaceutical Agents to Treat Endometriosis

The invention provides a method for the treatment of endometriosis. This condition is known to be mediated at least in part by oxidative stress and reactive oxygen species. Antioxidant compounds play important roles in ameliorating reactions leading to endometriosis. Thus, the probucol esters of the present invention, as anti-oxidants, are useful in the treatment of endometriosis.

Probucol Esters as Pharmaceuticals: Formulations and Modes of Delivery

The probucol esters of the invention can be formulated as pharmaceuticals for administration in a variety of ways. Typical routes of administration include both enteral and parenteral. These include, *e.g*, without limitation, subcutaneous, intramuscular, intravenous, intraperitoneal, intramedullary, intrapericardiac, intrabursal, intradermal, oral, sublingual, ocular, nasal, topical, transdermal, transmucosal, intrathecal, or rectal. The mode of administration can be, *e.g.*, via swallowing, inhalation, injection or topical application to a

surface (*e.g.*, eyes, mucous membrane, skin). Particular formulations typically are appropriate for specific modes of administration. Various contemplated formulations include, *e.g.*, aqueous solution, solid, aerosol, liposomal and transdermal formulations. Details on techniques for formulation and administration are well described in the scientific and patent literature, see, *e.g.*, the latest edition of "Remington's Pharmaceutical Sciences" (Maack Publishing Co, Easton PA).

Aqueous Solutions for Enteral, Parenteral Or Transmucosal Administration

Examples of aqueous solutions that can be used in formulations for enteral, parenteral or transmucosal drug delivery include, *e.g.*, water, saline, phosphate buffered saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions and the like. The formulations can contain pharmaceutically acceptable auxilliary substances to enhance stability, deliverability or solubility, such as buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. Additives can also include additional active ingredients such as bactericidal agents, or stabilizers. For example, the solution can contain sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate or triethanolamine oleate. These compositions can be sterilized by conventional, well-known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration.

Aqueous solutions are appropriate for injection and, in particular, for intravenous injection. The intravenous solution can include detergents and emulsifiers such as lipids. Aqueous solutions also are useful for enteral administration as tonics and administration to mucous or other membranes as, e.g., nose or eye drops. In one embodiment, the pharmaceutical composition of the invention contains a probucol ester in an amount of about 1 mg/ml to about 100 mg/ml, more preferably about 10 mg/ml to about 50 mg/ml.

Formulations For Enteral Or Transdermal Delivery

Solid formulations can be used for enteral administration. They can be formulated as, *e.g.*, pills, tablets, powders or capsules. For solid compositions, conventional nontoxic solid carriers can be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10%-95% of active ingredient.

A non-solid formulation can also be used for enteral (oral) administration. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, *e.g.*, peanut oil, soybean oil, mineral oil, sesame oil, and the like. See Sanchez, *et al.*, U.S. Patent No. 5,494,936.

Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like.

A unit dosage form, such as a tablet, can be between about about 50 mg/unit to about 2 grams/unit, preferably between about 100 mg/unit to about 1 gram/unit.

Topical Administration of Probucol Esters For Transdermal/ Transmucosal Delivery

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation. Such penetrants are generally known in the art, and include, e.g., for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents can be used to facilitate permeation. Transmucosal administration can be through nasal sprays, for example, or using suppositories.

For topical administration, the agents are formulated into ointments, creams, salves, powders and gels. In one embodiment, the transdermal delivery agent can be DMSO. Transdermal delivery systems can also include, *e.g.*, patches. Topical administration is particularly useful in the treatment of wounds. Where the treatment is localized (as for inducing healing of a wound), a pharmaceutical composition comprising, *e.g.*, hyaluronic acid, can be administered in the appropriate pharmaceutically acceptable formulation and administered topically. See, e.g., Polarek et al., *Wounds: a Compendium of Clinical Research and Practice*, 6:46-53 (1994).

The probucol esters can also be administered in sustained delivery or sustained release mechanisms, which can deliver the formulation internally. For example, biodegradeable microspheres or capsules or other biodegradeable polymer configurations capable of sustained delivery of a composition (*e.g.*, a probucol ester pharmaceutical) can be included in the formulations of the invention (see, *e.g.*, Putney (1998) *Nat. Biotechnol.* 16:153-157).

Probucol Ester Formulation Delivery By Inhalation

For inhalation, the probucol ester formulation can be delivered using any system known in the art, including dry powder aerosols, liquids delivery systems, air jet nebulizers, propellant systems, and the like. See, *e.g.*, Patton (1998) *Biotechniques* 16:141-143; inhallation delivery systems by, *e.g.*, Dura Pharmaceuticals (San Diego, CA) , Aradigm (Hayward, CA), Aerogen (Santa Clara, CA), Inhale Therapeutic Systems (San Carlos, CA), and the like.

For example, the pharmaceutical formulation can be administered in the form of an aerosol or mist. For aerosol administration, the formulation can be supplied in finely divided form along with a surfactant and propellant. The surfactant preferably is soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride such as, for example, ethylene glycol, glycerol, erythritol, arabitol, mannitol, sorbitol, the hexitol anhydrides derived from sorbitol, and the polyoxyethylene and polyoxypropylene derivatives of these esters. Mixed esters, such as mixed or natural glycerides can be employed. The surfactant can constitute 0.1%-20% by weight of the composition, preferably 0.25%-5%. The balance of the formulation is ordinarily propellant. Liquefied propellants are typically gases at ambient conditions, and are condensed under pressure. Among suitable liquefied propellants are the lower alkanes containing up to 5 carbons, such as butane and propane; and preferably fluorinated or fluorochlorinated alkanes. Mixtures of the above can also be employed. In producing the aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve. See, *e.g.*, Edwards (1997) *Science* 276:1868-1871.

A nebulizer or aerosolizer device for administering probucol esters of this invention typically delivers an inhaled dose of about 1 mg/m^3 to about 50 mg/m^3.

Delivery by inhalation is particular effective for delivery to respiratory tissues for the treatment of respiratory conditions including an inflammatory component.

Other Formulations

In preparing pharmaceuticals of the present invention, a variety of formulation modifications can be used and manipulated to alter pharmacokinetics and biodistribution. A number of methods for altering pharmacokinetics and biodistribution are known to one of ordinary skill in the art. Examples of such methods include protection of the complexes in vesicles composed of substances such as proteins, lipids (for example, liposomes), carbohydrates, or synthetic polymers (discussed above). For a general discussion of pharmacokinetics, *See, Remington's Pharmaceutical Sciences, supra*, Chapters 37-39.

Administration

The methods of the invention inhibit oxidation and oxidative damage in a subject and treat or prevent a variety of injuries, diseases and conditions, including, *e.g.*, viral infections, reperfusion injury, restenosis, hypercholesterolemia, peripheral and central neuropathy, opthalmic disorders, pulmonary disorders, cardiovascular disease, diabetic neurovascular pathology, eclampsia, and conditions caused or exacerbated by oxidative stress. The amount of probucol ester adequate to accomplish this is defined as a "pharmacologically effect amount" (see definitions, above). The dosage schedule and amounts effective for this use, *i.e.*, the "dosing regimen," will depend upon a variety of factors, including frequency of dosing, the stage of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient's physical status, age and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration.

The dosage regimen must also take into consideration the pharmacokinetics, *i.e.*, the probucol ester's rate of absorption, bioavailability, metabolism, clearance, and the like (see, *e.g.*, Komura (1997) "Effect of LDL-apheresis on the pharmacokinetics of the lipophilic antilipidemic agent probucol," *Eur. J. Drug Metab. Pharmacokinet.* 22:201-206;

Hidalgo-Aragones (1996) *J. Steroid Biochem. Mol. Biol.* 58:611-617; Groning (1996) *Pharmazie* 51:337-341; Johnson (1995) *J. Pharm. Sci.* 84:1144-1146; Rohatagi (1997) *Pharmazie* 52:529-532; the latest Remington's edition, *supra*). The hydrolysis rate of the producol ester and the pharmacokinetics of the hydrolysis product are also important considerations.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of a probucol ester sufficient to treat the patient effectively. The total effective amount of a probucol ester of the present invention can be administered to a subject as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which the multiple doses are administered over a more prolonged period of time. One skilled in the art would know that the concentration of a probucol ester of the present invention required to obtain an effective dose (*i.e.*, a "therapeutically effective dose") in a subject depends on many factors including, *e.g.*, the pharmacokinetics of the prodrug and of its hydrolysis product, the age and general health of the subject, the route of administration, the number of treatments to be administered and the judgment of the prescribing physician. In view of these factors, the skilled artisan would adjust the dose so as to provide an effective dose for a particular use.

Probucol Esters in Bio-Organic Materials

The invention provides a composition comprising a bio-organic material and an amount of a probucol ester of the invention effective to inhibit oxidation of the bio-organic material. The bio-organic material can be blood plasma, nutrient media, protein, a pharmaceutical, a cosmetic, a sperm or oocyte preparation, cells, cell cultures, viruses, foods, drinks, implant materials or implantable devices (*e.g.*, plastics, artificial heart valves or joints, collagens), medical materials (*e.g.*, tubing for catheterization, intubation, IVs) and containers (*e.g.*, blood bags, storage containers), and the like. The composition can contain a mixture of different probucol esters of the invention (*e.g.*, probucol monophosphate, probucol diphosphate, or diphospho-probucol) in varying amounts.

EXAMPLES

The following examples are offered to illustrate, but not to limit, the claimed invention.

Example 1: Synthesis of Probucol Phosphate Esters

The following example details the synthesis of the probucol monophosphate and probucol diphosphate esters of the invention.

A volume of 1.24 ml of 2.5 M butyl-lithium solution in hexane was added dropwise to a stirred solution of 1.5 mmol probucol in 50 ml of ether, under argon atmosphere. To the resulting suspension was slowly added 3.1 mmol phosphorous oxychloride solution in 10 ml of ether. The mixture was then treated with 5 ml water, followed by 50 ml of 2M potassium hydroxide. The ether phase was separated and discarded. The aqueous phase was adjusted to pH 8.5 and washed with 1:1 ethyl acetate:hexane to remove neutral products. The pH was then adjusted to 4.25 and the solution was extracted with 1:1 ethyl acetate:hexane. The organic phase contained mostly (more than 50%) probucol monophosphate and the aqueous phase contained mostly probucol diphosphate, as analyzed by HPLC.

Synthesis of Probucol Monophosphate Free Acid

The organic phase was washed first with 1M phosphate buffer, pH 4.5, and then with brine-HCl. After drying over sodium sulfate and removing (evaporating) volatile solvents, probucol monophosphate (free acid) was obtained as a solid residue, weighing 0.15 gm, 16.2% yield. The product was characterized by electrospray mass spectroscopy and nuclear magnetic resonance spectroscopy.

Electrospray analysis: MS (negative mode) m/z 595 $[M-H]^-$.

Proton magnetic resonance spectroscopy: ^{1}H-NMR (500 MHz, DMSO-d_6), delta: 1.376-1.426 (two s, 42H, all methyl groups), 7.343 (s, 2H, C_3-H, C_5-H). Phosphorous magnetic resonance spectroscopy: ^{31}P-NMR (500 MHz, DMSO-d_6) delta: -5.973.

Synthesis of Probucol Monophosphate Sodium Salt

One equivalent of the monophosphoric acid ester was dissolved in isopropyl alcohol and treated with one equivalent of 1M sodium hydroxide. The solvent was removed under vacuum and the residue was again dissolved in isopropyl alcohol, filtered and freed of solvent. Probucol monophosphate sodium salt was obtained in quantitative yield as an amorphous residue and characterized by electrospray mass spectroscopy and nuclear magnetic resonance spectroscopy.

Electrospray analysis: MS m/z 619 $[M+H]^+$. Proton magnetic resonance spectroscopy: ^{1}H-NMR (500 MHz, DMSO-d_6), delta: 1.377-1.452 (two s, 42H, all methyl groups), 7.332 (s, 2H, $C_{3'}$-H, $C_{5'}$-H), 7.359 (s, 2H, C_3-H, C_5-H). Phosphorous magnetic resonance spectroscopy: ^{31}P-NMR (500 MHz, DMSO-d_6) delta: -3.341.

Synthesis of Probucol Diphosphate Free Acid

The aqueous phase from the initial reaction mixture described above was washed with ethyl acetate to remove any remaining (contaminating) probucol monophosphate. The pH was then adjusted to 2 and the solution extracted with ethyl acetate. The extract was washed with brine, dried over sodium sulfate and freed of solvent under vacuum. Probucol diphosphate (free acid) was obtained as a solid residue, 0.35 g, 33.4% yield. The product was characterized by electrospray mass spectroscopy and nuclear magnetic resonance spectroscopy.

Electrospray analysis: electrospray MS m/z 677 $[M+H]^+$, 699 $[M+Na]^+$. Proton magnetic resonance spectroscopy: ^{1}H-NMR (500 MHz, DMSO-d_6), delta: 1.428 (s, 42H, all methyl groups), 7.492 (s, 4H, all aromatic protons). Phosphorous magnetic resonance spectroscopy: ^{31}P-NMR (500 MHz, DMSO-d_6), delta: -5.882.

Synthesis of Probucol Diphosphate Disodium Salt

One equivalent of the diphosphoric acid ester was treated with two equivalents of 1M sodium hydroxide. The solvent was removed under vacuum, and the residue was washed with warm isopropyl alcohol and dried under vacuum. The product obtained in quantitative yield was characterized by electrospray mass spectroscopy and nuclear magnetic resonance spectroscopy.

Electrospray analysis: MS m/z 721 $[M+H]^+$, 743 $[M+Na]^+$. Proton magnetic resonance spectroscopy: ^{1}H-NMR (500 MHz, DMSO-d_6), delta: 1.453 (s, 42H, all methyl groups), 7.377 (s, 4H, all aromatic protons). Phosphorous magnetic resonance spectroscopy: ^{31}P-NMR (500 MHz, DMSO-d_6), delta: -3.895.

Synthesis of Diprobucol Phosphate Free Acid

Also isolated in low yield from the phosphorylation reaction mixture described above was the di(probucol) ester of phosphoric acid, which was characterized by electrospray mass spectroscopy and nuclear magnetic resonance spectroscopy.

Example 2: Probucol Phosphate Esters as Antioxidants in Cells

The following example details the use of the probucol monophosphate and probucol diphosphate esters of the invention as antioxidants; and, using an *in vitro* cell culture model, demonstrates that they are effective as antioxidants in a biological system.

Experiments were conducted with smooth muscle cells, endothelial cells and monocytes. Diprobucol monophosphate and probucol monophosphate significantly inhibited oxidation of low density lipoproteins (LDLs) in tests using the above cell lines. No such inhibition was detected when probucol was used. Thus, diprobucol monophosphate and probucol monophosphate were able to permeate the cells, whereas probucol was not.

Example 3: Probucol Esters Protecting Against LDL Oxidation by Copper and Horseradish Peroxidase/ Hydrogen Peroxide.

The following example describes the use of the probucol monophosphate and probucol diphosphate esters of the invention as antioxidants in the protection of LDL aginast oxidation by copper and by horseradish peroxidase and hydrogen peroxide.

Copper ions (in the presence of air) and horseradish peroxidase/hydrogen peroxide are both known to cause oxidation of LDL (Proudfoot (1997) *Free Radic. Biol. Med.* 23:699-705). The damage to LDL can be monitered by measuring the increase in ultraviolet absorption at 234 nm due to the formation of conjugated dienes. The experimental methodology and the sources of LDL, horseradish peroxidase and other materials are described by Santanam (1997) *FEBS Letters* 414:549-551.

Under the aformentioned conditions, LDL in the presence of horse radish peroxidase/hydrogen peroxide exhibited a characteristic time course of oxidation, with a lag phase of about two hours followed by an onset of oxidation that became maximal after about five hours. The addition of 5 uM probucol or 5 uM probucol monophosphate delayed the lag phase and completely eliminated the onset of oxidation to about seven hours.

These results demonstrate that probucol and probucol monophosphate are potent antioxidants. They also demonstrate that probucol diphosphate is an effective antioxidant, even in the absence of enzymes needed to convert the diphosphate probucol to its hydrolysis products probucol monophosphate and probucol.

Similar results were obtained with the copper-mediated oxidation of LDL.

Example 4: Probucol Esters Inhibiting Oxidation by Myeloperoxidase.

The following example details the use of the probucol diphosphate ester of the invention to prevent oxidation by the enzyme myeloperoxidase.

The protective effect of probucol diphosphate on myeloperoxidase (MPO) oxidation of 1.4 mM tetramethylbenzidine was assayed by measuring the oxidation product at 665 nm. The reagents used were myeloperoxidase from human leukocytes (cat. #M6908, Sigma, St. Louis, MO) and 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMBD) (cat. #86151-0, Aldrich Chemical, Milwaukee, WI). MPO (0.025 standard unit) and TMBD were incubated at room temperature in acetate buffer (50 mM, pH 5.4) containing 8% of N,N-dimethyl formamide (DMF) and various concentration of probucol diphosphate (5 uM, 10 uM, 20 uM, 30 uM and 40 uM). The reaction was started by adding 300 uM H_2O_2, stopped by adding 10 ug/mL of catalase. The reaction mixture was diluted with 0.2M acetic acid in the ratio 1:1 and then the UV spectra were measured.

Probucol diphosphate completely inhibited MPO as compared to probucol, which did not show any inhibition effect of MPO activity in this test

Example 5: Stability of Probucol Esters In Aqueous Solutions.

The following example details the stability of the probucol esters of the invention in an aqueous solution.

Probucol diphosphate was dissolved in a 1:1 mixture of 0.1 M sodium phosphate buffer and ethanol. Ethanol is ued as a cosolvent in order to help in the dissolution of probucol monophosphate and probucol, and to maintain solution clarity. Aliquots were adjusted to pH 1, 4, 6, 7.4 and 14, then maintained at 37°C for several time periods, as outlined below. Analyses for probucol, probucol monophosphate and probucol diphosphate were performed by thin layer chromatography.

The estimated half-life for hydrolysis of probucol diphosphate ester to probucol monophosphate and free (unphosphorylated) probucol was calculated using the data obtained from this experiment:

	Estimated Half-Life for Hydrolysis to:	
pH	Free Probucol	Probucol Monophosphate
1	---	3 weeks
4	2 weeks	15 hours
6	2 weeks	15 hours
7.4	4 weeks	30 hours
14	Not measurable	Not measurable

Example 6: **Enzymatic Hydrolysis of Probucol Esters**

The following example describes the enzymatic conversion of probucol diphosphate to probucol monophosphate in an aqueous solution.

Alkaline phosphatase.

Probucol diphosphate disodium salt (2 mg) and 5 units of alkaline phosphatase (E. coli, type III, Sigma cat. #P4252) were dissolved in 5 ml of glycine buffer, pH 10.4, containing 1 mM $MgSO_4$ and 0.2 mM $ZnSO_4$. After 20 hours at 37°C, analysis by standard thin layer chromatography showed the presence of 6% probucol monophosphate. These results demonstrate that probucol diphosphate is enzymatically hydrolyzed by alkaline phosphatase.

Acid phosphatase

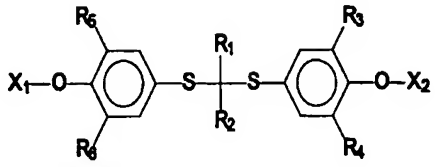
Probucol diphosphate disodium salt (2 mg) and 5 units of acid phosphatase (Potato, type III, Sigma cat. #P6760) were dissolved in 5 ml of 50 mM citrate buffer, pH 4.8, containing 1 mM $MgCl_2$. After 20 hours at 37°C, the solution was milky, and analysis by thin layer chromatography showed the presence of 60% probucol monophosphate as the only product. The same results were obtained with Potato acid phosphatase Type XA (Sigma cat.

#P1435). These results demonstrate that probucol diphosphate is enzymatically hydrolyzed by acid phosphatase.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A probucol ester of the formula selected from the group consisting of:



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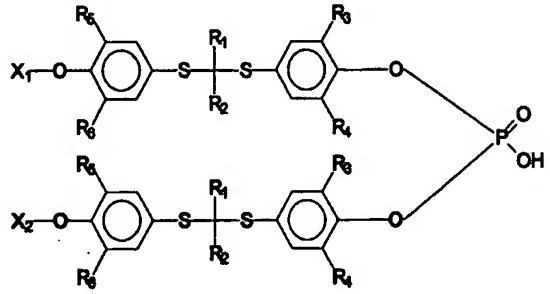
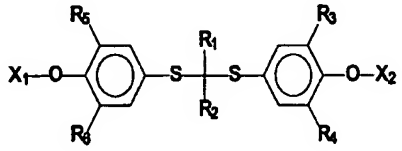


wherein X_1 is H- or - PO_3-H_2, and X_2 is H- or - PO_3-H_2 ; R_1 and R_2 are independently selected from H- or -CH_3; and R_3, R_4, R_5, and R_6 are independently selected from H- or an alkyl group selected from the group consisting of methyl, ethyl, propyl, butyl and tert-butyl; and,

a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein R_3, R_4, R_5, and R_6 are tert-butyl groups.

3. A compound of the formula:

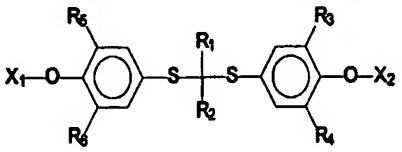


wherein X_1 is a lithium, and X_2 is H- or a lithium; R_1 and R_2 are independently selected from H- or -CH_3; and R_3, R_4, R_5, and R_6 are independently selected from H- or an alkyl group selected from the group consisting of methyl, ethyl, propyl, butyl and tert-butyl; and

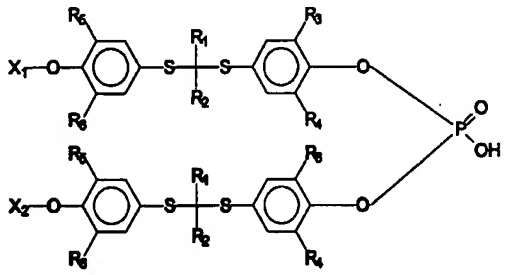
a pharmaceutically acceptable salt thereof.

4. The compound of claim 2, wherein R_3, R_4, R_5, and R_6 are tert-butyl groups.

5. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a pharmacologically effective amount of a compound selected from the group consisting of



,



wherein X_1 is H- or - PO_3-H_2, and X_2 is H- or - PO_3-H_2 ; R_1 and R_2 are independently selected from H- or -CH_3; and R_3, R_4, R_5, and R_6 are independently selected from H- or an alkyl group selected from the group consisting of methyl, ethyl, propyl, butyl and tert-butyl; and,

a pharmaceutically acceptable salt thereof.

6. The pharmaceutical composition of claim 5, wherein R_3, R_4, R_5, and R_6 are tert-butyl groups.

7. The pharmaceutical composition of claim 5, wherein the pharmaceutically acceptable carrier is an aqueous solution, a solid, a liposome, a transdermal carrier, a detergent or an emulsifier.

8. The pharmaceutical composition of claim 5 in a unit dosage form.

9. The pharmaceutical composition of claim 8 wherein the unit dosage form delivers between about 0.1 mg per Kg to about 1 gm per Kg of the compound.

10. The pharmaceutical composition of claim 9 wherein the unit dosage form delivers between about 1.0 mg per kg to about 100 mg per kg of the compound.

11. The pharmaceutical composition of claim 10 wherein the unit dosage delivers between about 10 mg per kg to about 50 mg per kg.

12. The pharmaceutical composition of claim 8 wherein unit dosage form for administration is in the form of a powder, a tablet, a capsule or a lozenge.

13. The pharmaceutical composition of claim 5 in the form of an injectable solution.

14. The pharmaceutical composition of claim 13 wherein the concentration of the compound in the injectable solution is between about 1 mg/ml to about 100 mg/ml.

15. The pharmaceutical composition of claim 5 contained in an aerosolizer or inhaler.

16. The pharmaceutical composition of claim 5 contained in a transdermal delivery system.

17. A method for inhibiting oxidation in a biological material comprising administering to the material the compound of claim 1 in an amount effective to inhibit oxidation.

18. The method of claim 17, wherein the biological material is a biological solution, a cell or a tissue.

19. The method of claim 18, wherein the cell or tissue is derived from a plant, an animal, or a human.

20. A method for inhibiting oxidation in an organism comprising administering to the organism the compound of claim 1 in an amount effective to inhibit the oxidation.

21. The method of claim 20, wherein the organism is a human.

22. The method of claim 20, wherein the effective amount is about 1.0 mg per kg to about 1 gm per kg.

23. The method of claim 20, wherein the pharmaceutical composition is administered intramuscularly, intravenously, intraperitoneally, intrathecally, orally, rectally, nasally, ocularly, transdermally, topically or as an inhalant.

24. The method of claim 20, wherein the oxidation is mediated by an enzyme.

25. A method for the treatment of a condition with an inflammatory component in a subject, comprising administering to the subject the compound of claim 1 in an amount effective to treat the condition.

26. The method of claim 25, wherein the compound is administered in the form of an intravenous injection of a pharmaceutical composition comprising the compound and a pharmaceutically acceptable aqueous carrier.

27. The method of claim 26, wherein the concentration of the compound in the pharmaceutical composition is between about 0.1 mg/ml to about 100 mg/ml.

28. The method of claim 27, wherein the concentration of the compound in the pharmaceutical composition is between about 1 mg/ml to about 20 mg/ml.

29. The method of claim 25, wherein the condition with an inflammatory component is an arthritis.

30. The method of claim 29, wherein the arthritis is rheumatoid arthritis, psoriatic arthritis, or osteoarthritis.

31. The method of claim 25, wherein the condition with an inflammatory component is a peripheral neuropathy.

32. The method of claim 25, wherein the condition with an inflammatory component is a pathologic condition or injury of the central nervous system.

33. The method of claim 32, wherein the central nervous system condition is beta-amyloid neurotoxicity.

34. The method of claim 33, wherein the beta-amyloid neurotoxicity is Alzheimer's Disease.

35. A method for the treatment of a viral infection in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the viral infection.

36. The method of claim 35, wherein the viral infection is selected from the group consisting of a herpes virus infection, a cytomegalovirus infection, an Epstein Barr virus infection, a varicella zoster virus infection, a Marek's disease infection, and a retroviral infection.

37. The method of claim 35, wherein the viral infection is a retroviral infection.

38. The method of claim 37, wherein the retroviral infection is selected from the group consisting of an HIV-1 infection, an HIV-2 infection, an HTLV-1 infection, and an HTLV-2 infection.

39. The method of claim 35, wherein the compound is applied topically.

40. A method for the treatment of a viral infection in a subject comprising administering to the subject a pharmacologically effective amount of a compound of claim 1, wherein said administering to the subject comprises the ex vivo treatment of cells.

41. The method of claim 40, wherein the cells comprise lymphocytes.

42. A product of manufacture for the treatment of a viral infection in a subject comprising a pharmacologically effective amount of a compound of claim 1.

43. The product of manufacture of claim 42, wherein the product is a lubricant for sexual intercourse.

44. The product of manufacture of claim 43, wherein the product comprises a prophylactic device.

45. A method for the treatment of reperfusion injury in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the reperfusion injury.

46. The method of claim 45, wherein the reperfusion injury is caused by cerebrovascular accident, stroke, myocardial infarction, or trauma.

47. A method for the treatment of restenosis in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the restenosis.

48. The method of claim 47, wherein the restenosis occurs after angioplasty, coronary bypass surgery, cardiovascular surgery or fibrolytic procedures.

49. A method for the treatment of hypercholesterolemia in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the hypercholesterolemia.

50. A method for inhibiting the oxidation of low density lipoproteins in a subject, comprising administering to the subject the compound of claim 1 in an amount effective to inhibit the oxidation.

51. A method for the treatment of an opthalmic disorder in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the opthalmic disorder.

52. The method of claim 51, wherein the opthalmic disorder is macular degeneration.

53. A method for the treatment of a hearing loss in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the hearing loss.

54. The method of claim 53, wherein the hearing loss is noise-induced hearing loss.

55. A method for the treatment of a pulmonary disorder in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the pulmonary disorder.

56. The method of claim 55, wherein the pulmonary disorder is asthma, acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), oxygen exposure or cystic fibrosis.

57. A method for the treatment of a cardiovascular disease in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the cardiovascular disease.

58. The method of claim 57, wherein the cardiovascular disease is atherosclerosis, cerebrovascular accident, stroke, restenosis, ischemia injury, reperfusion injury, and myocardial infarction.

59. A method for inhibiting cholesterol oxide formation in a subject comprising administering to the subject the compound of claim 1 in an amount effective to inhibit cholesterol oxide formation.

60. A method for the inhibition of macrophage foam cell formation in a subject comprising administering to the subject the compound of claim 1 in an amount effective to inhibit macrophage foam cell formation.

61. A method for the inhibition of oxysterol synthesis in a subject comprising administering to the subject the compound of claim 1 in an amount effective to inhibit oxysterol synthesis.

62. The method of claim 61, wherein the oxysterol is 7 beta-hydroxycholesterol or 7-ketocholesterol.

63. A method for the treatment of diabetes mellitus in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the diabetes mellitus.

64. A method for the treatment of a drug-induced oxidative stress in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the drug-induced oxidative stress.

65. The method of claim 64, wherein the drug is a chemotherapeutic agent or an antitumor drug.

66. The method of claim 65, wherein the chemotherapeutic agent is bleomycin or adriamycin.

67. A method for the treatment of a cancer in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the cancer.

68. A method for the treatment of pre-eclampsia and eclampsia in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the pre-eclampsia or eclampsia.

69. A method for the treatment of endometriosis in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the endometriosis.

70. A method for the treatment of infertility due to increased oxidative stress in a subject comprising administering to the subject the compound of claim 1 in an amount effective to treat the infertility.

71. A composition comprising a bio-organic material and a compound of claim 1 in an amount effective to inhibit oxidation of the bio-organic material.

72. The composition of claim 71, wherein the bio-organic material is a blood plasma preparation, a nutrient media, a protein, a pharmaceutical, a cosmetic, a sperm or a oocyte preparation, cells or a cell culture, a virus, a food, a drink, an implant material or an implantable device, a medical materials or a container for bio-organic material.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/23746

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07C 319/00; C07C 9/02
US CL :568/39, 40; 558/162, 197

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 568/39, 40; 558/162, 197

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS file Registry STRUCTURE Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	Chem. Abstr. Vol. 73, 1970 (Columbus, OH, USA) Abstract No. 45047, NEUWORTH, M. ET AL. 'Synthesis and Hypocholesterolemic activity of alkylidene dithio bisphenols.' J. Med. Chem. (1970) 13(4) 722-725.	1, 2, 4, 5, 6, 7-72 ---------- 3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 FEBRUARY 1999	Date of mailing of the international search report 16 MAR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PAUL J. KILLOS aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★